299-Pos Board B79
The Effect of Crystal Contact Forces on Protein Intramolecular Dynamics
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Increasingly time resolved X-ray crystallography and solid state NMR have been employed to characterize dynamics. In the advent of X-ray free electron sources at Stanford (LCLS), and Hamburg (European XFEL) there is a strong push to extend time-resolved measurements. A persistent question for these techniques however, is how the crystal contact forces may strongly perturb these dynamics from those in vivo. While some theoretical studies have indicated that the crystal contact perturbation is minor[1], other calculations suggest it is significant[2]. Surprisingly there have been few studies to actually determine from the data what the effects are. Given the enormous effort currently underway for extending crystal phase dynamics measurements, it is imperative to determine how the crystal contact forces affect large scale motions necessary for function. Here we show how anisotropic optical measurements in the extreme infrared (10-100 cm-1) using the technique of Crystal Anisotropy Terahertz Microscopy (CATM) can quantify the effect [3], by measuring the perturbation of the global motions for a given symmetry group. Chicken egg white lysozyme (CEWL) is used as a benchmarking model. Calculations and measurements are performed for tetragonal and monoclinic symmetry groups, for which B-factor measurements indicate that there is a significant difference in the motional constraint arising from the crystal geometry.
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300-Pos Board B80
Thermodynamic and Dynamic Basis for the Broadened Ligand Specificity of a Tiam2 PDZ Domain Mutant
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PDZ (PSD-95/Dlg/ZO-1) domains are protein-protein interaction modules that typically recognize their binding partners through the use of two specificity pockets. Here we examine the consequence of mutating four residues in the Tiam2 PDZ domain specificity pockets to produce a quadruple mutant (QM). Equilibrium binding studies show that the specificity of the Tiam2 QM mutant is similar to that seen in the wild type Tiam1 PDZ domain. Isothermal titration calorimetry experiments show a larger entropic contribution to ligand binding in the QM PDZ domain compared to the WT PDZ domain. Double-mutant cycle analysis uncovered cooperativity between residues in the two specificity pockets with respect to both ligand binding and protein folding. NMR-based HSCQ studies reveal that the wild type Tiam2 PDZ has severe line broadening in several loop regions, while the QM PDZ had additional regions of line broadening. However, peptide ligand binding dampens line broadening for both the Tiam2 WT and QM PDZ domains. Finally, CPMG dispersion experiments indicate that the number of residues experiencing micro to millisecond motions is significantly increased in the QM PDZ domain. We propose a model where enhanced dynamics alters the QM PDZ domain conformational ensemble allowing for broader ligand specificity relative to the WT PDZ domain.

301-Pos Board B81
Relative Mechanical Flexibility of Ubiquitin Family Proteins: A Study using Elastic Network Model
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The conformational flexibility of biomolecules is essential for their function. Elastic Network Model (ENM) is a class of harmonic models used to computationally describe the flexibility of biomolecules. Despite the simplicity of the underlying potential, ENMs show intriguing abilities to capture functionally relevant conformational changes in proteins, as seen in their crystallographic structures, through their low-frequency normal-mode displacements. We present an ENM based study of the mechanical flexibility of proteins having high structural similarity but low sequence homology.
Single-molecule atomic force microscopic (AFM) measurements reveal that ubiquitin requires a higher unfolding force when pulled along N-C termini than the SUMO proteins. The higher mechanical stability of ubiquitin relative to the SUMOs is presumably a sequence effect, as the proteins have identical secondary structures. Our calculations at the atomistic resolution show a strong imprint of the experimentally observed disparity in stabilities of the ubiquitin-like proteins in their flexibilities. Spring constants for normal modes of ubiquitin are higher than that of the SUMOs, implying larger stiffness of ubiquitin over the latter. The residues on the clamp (terminal β-sheets) region of these proteins that govern their stabilities show mobility that is implicated in their flexibilities. We discuss physical considerations for extracting a reduced dimensional basis from ENM for the description of equilibrium flexibility of proteins.
The large-amplitude normal modes that represent concerted protein motions additionally reveal the conformational changes taking place when ubiquitin and SUMOs bind with substrates, as observed in the complex crystallographic structures. The flexible SUMO proteins tend to be as stiff as ubiquitin on substrate-binding whereas, there seems to be no considerable enhancement in the rigidity of apo-ubiquitin. We elucidate this feature in our study in the light of spring constants of the slowest normal modes.

302-Pos Board B82
Motion and Conformational Entropy in Protein Function: Creation of an NMR-Based Entropy Meter
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Conformational entropy is a potentially important thermodynamic parameter contributing to protein function. Quantitative measures of conformational entropy are necessary for an understanding of its role but have been difficult to obtain. We have recently introduced empirical method that utilizes changes in conformational dynamics as a proxy for changes in conformational entropy. We have now used molecular dynamics simulations to probe the microscopic origins of the link between conformational dynamics and conformational entropy. Simulation of seven proteins gave an excellent correlation with measures of side-chain motion derived from NMR relaxation. The simulations show that the motion of methyl-bearing side-chains are sufficiently coupled to that of other side chains to serve as excellent reporters of the overall side-chain conformational entropy. These results tend to validate the use of experimentally accessible measures of methyl motion - the NMR-derived generalized order parameters - as a proxy from which to derive changes in protein conformational entropy due to a perturbation such as the binding of a ligand. A slightly modified weighting scheme to project the change in dynamics of experimental methyl dynamics into conformational entropy is presented. Originally based on data from the calmodulin system, we will describe experimental results from other systems that indicate that the "entropy meter" approach is both robust and general and that the involved conformational entropies are often large and cannot be ignored. Supported by the NIH and the Mathers Foundation.

303-Pos Board B83
A Tool Set to Map Dynamic Allosteric Networks through the NMR Chemical Shift Covariance Analysis (CHESCA)
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Allostery is an essential regulatory mechanism of biological function and allosteric sites are also pharmacologically relevant, as they are typically targeted with higher selectivity than orthosteric sites. However, obtaining a comprehensive map of allosteric sites poses experimental challenges because allostery is driven not only by structural changes, but also by modulations in dynamics that often remain elusive to classical structure determination methods. An avenue to overcome these challenges is provided by the covariance analysis of NMR chemical shift [1, 2], which are exquisitely sensitive to redistributions in dynamic conformational ensembles. Here, we propose a set of complementary algorithms for the NMR chemical shift covariance analysis (CHESCA) designed to reliably detect allosteric networks with minimal occurrences of false positives or negatives. The proposed CHESCA toolset was tested for two allosteric proteins (Protein Kinase A, PKA, and the Exchange Protein directly Activated by cAMP, EPAC) and is expected to complement traditional comparative structural analyses in the comprehensive identification of functionally relevant allosteric sites, including those in otherwise elusive partially unstructured regions.
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304-Pos Board B84
Probing Multiple Timescale Dynamics of Protein Kinase A-Inhibitor Complexes
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